

SOME EFFECTS OF THE OCCURRENCE OF *MYXOSPORIDIA* IN THE GALL BLADDER OF FISHES

(PRELIMINARY COMMUNICATION)

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I. INTRODUCTION

Recently, while working on various protozoal parasites of marine fishes, occasion was made to investigate the more direct effects of these parasites upon the hosts harbouring them. The present paper is a short account of some results obtained when studying the effects of certain *Myxosporidia* inhabiting the gall bladders of various fishes.

II. MATERIAL

The fish in question harboured species of *Myxidium*, *Ceratomyxa*, *Chloromyxum* and *Leptotheca*. Mixed infections of

Myxosporidia occasionally occurred, but the effect of such co-existences was the same as that of either factor considered separately, and so is included here.

The hosts were examined as soon as possible after the fish were taken. Frequently they were killed for the purpose of examination. Our results have been arranged so as to exclude any post-mortem effects. The fish were opened rapidly and the contents of the gall bladders siphoned off, as a rule. The quantity of bile could be thus estimated, as well as its fluidity or viscosity. Sizes of fish—judged usually by length as a better criterion of age than weight, though the latter was also considered—were kept for purposes of comparison. It is to be regretted that more published data as to the normal variation in size and weight of fishes of the same age are not available.

The examinations here recorded extended over five weeks, during which time we were the guests of the Laboratoire Maritime at Luc-sur-mer, Normandy, through the kindness of the Director, Professor Joyeux-Laffaie, to whom we return cordial thanks. We would especially thank heartily Dr. C. Lebailly, who was in charge of the Laboratory, and who spared neither time nor trouble in procuring us materials for research. We also are indebted to Mr. Tate Regan, of the British Museum, for kindly naming some of the fishes (young *Raja*).

III. EFFECTS ON BILE AND GALL BLADDER

Our work embraced both *Elasmobranchii* and *Teleostei*, and we propose to tabulate the results obtained in both groups. The fish investigated is given a serial number and the degree of infection with *Myxosporidia* is indicated. A brief statement as to the colour and density of the bile, and one on the condition of the gall bladder, follows. Thin-walled gall bladders were usually transparent, and only where the transparency was more noticeable than usual is this recorded in the tables.

As the majority of the fish examined (over 100) belong to the *Teleostei*, they are considered first, members of the same tribe of hosts being arranged together for convenience.

(A) TELEOSTEI

TABLE I.—*Gadus pollacchius* (= *Merlangus pollacchius*). Young fish.

No. of fish	Degree of infection with Myxosporidia	Condition of bile	Condition of gall bladder	Other remarks
1	Heavy	Yellow, thickish	Slightly thick walled	Fish weighed 350 grams.
2	Slight	Yellowish	"	
3	"	"	"	
4	"	Green	"	Weight 350 grams.
5	Uninfected	"	Transparent	" 350 "
6	"	Bright green	Large, thin	(These fish, except
7	"	"	Small, thin	No. 23, were 27 to 34 cm. long).
8	Slight	Yellowish green	"	
9	Uninfected	Greenish orange	"	Examined 5 hours after death.
10	Fair number of parasites	Yellowish orange, thick, turbid	Thick walled	Weight 340 grams.
11	Uninfected	Very pale yellow	Thin	" 370 "
12	Very slight infection	"	"	" 370 "
13	Many parasites	Yellow, thickish	Thick walled	" 360 "
14	Uninfected	Green	Very thin	" 370 "
15	Heavy infection	Yellow	Thick walled	" 350 "
16	"	Orange, thick	"	" 350 "
17	Uninfected	Greenish, very fluid	Thin, transparent	" 375 "
18	Very heavy infection	Brownish yellow	Large, thick walled	
19	Uninfected	Bright green	Thin walled	
20	"	"	"	
21	Slight infection	Yellow, thin	"	
22	Heavy "	Exceedingly thick, straw-coloured purée	Very thick walled indeed, small cavity	Weight 340 grams.
23	Uninfected	Very fluid, green	Thin walled	Large fish, weight 811 grams, length 46 cms.
24	"	Yellowish green, very fluid	Thin	Many worms present
25	Fairly heavy infection	Yellowish brown, lumps of spores in it	Thick, opaque	Weight 350 grams.
26	Very heavy infection	Little bile; very thick, straw-coloured purée	Thick, white, extremely tough, small cavity	Shortest fish of the series, 27 cm. long. Wt. 340 grams. Observed for 12 days.

From the above table it is seen that fifteen of the twenty-six *Gadus pollachius* examined were infected in their gall bladders by a species of *Myxosporidia*, the percentage of infection being 57.7. Five cases only could be described as heavy infections, a percentage of 19.2. Examination of the bile of the uninfected *Gadus pollachius* showed that in every case the colour predominating was green. Nine of the eleven uninfected fish had bile that was vivid green—almost moss-green—in colour. One case had greenish-orange bile, but here the results were possibly invalid, as the fish had been dead for five hours before it was examined. The bile of uninfected *Gadus pollachius* was also very limpid.

When *Myxosporidia* were present, the bile coloration underwent profound changes, and became yellow to orange, and even brown. When the parasites were present in very large numbers, the condition of the bile was one of high concentration, and the contents of the gall bladder resembled a thick, straw-coloured purée. As a general statement, the larger the number of parasites the more opaque, viscid and light-coloured was the bile.

The effect on the gall bladder appears to be no less marked. Ordinarily in uninfected fish it is thin-walled and transparent. With progressive infection it becomes thicker and its cavity smaller, while its opacity increases enormously. The condition is discussed in Section III (c), page 477.

TABLE 2.—*Gadus merlangus* (= *Merlangus vulgaris*).

No. of fish	Degree of infection with <i>Myxosporidia</i>	Condition of bile	Condition of gall bladder	Other remarks
1	Uninfected	Thin, green	Thin	Fish 26.5 cm. long
2	Moderate infection	Brownish yellow, thick	Thick	" " "
3	Uninfected	Fluid, limpid, green	Thin	" " "
4	Slight infection	Brownish yellow	Somewhat thicker	" " "

In this case, the percentage of infected fish was high, 50 per cent., but judging from other experiences, it is likely that a

different result would have been obtained had it been possible to examine more fish. The point of interest, however, that the *Myxosporidia* in the gall bladder induced thickening of its walls and alteration of the bile, remains the same as in the case of *Gadus pollachius*.

TABLE 3.—*Gadus luscus*.

No. of fish	Degree of infection with <i>Myxosporidia</i>	Condition of bile	Condition of gall bladder	Other remarks
1	Uninfected	Green	Thin walled	All these fish were young, and belonged to the same 'school.' They were about 16 cm. long.
2	"	Yellowish Green	"	
3	"	"	"	
4	"	Green	"	
5	"	"	"	
6	"	"	"	
7	"	"	"	
8	"	"	"	
9	"	"	"	
10	"	"	"	
11	"	"	"	
12	"	"	"	
13	"	"	"	
14	"	"	"	

No *Gadus luscus* examined harboured *Myxosporidia* in its gall bladder. In twelve cases out of fourteen the bile was of a bright green hue, and in the remaining two cases a faint yellowish tinge only was perceptible.

The *Gadidae*, as exemplified by *Gadus pollachius*, *Gadus merlangus* and *Gadus luscus*, appear to have normally green bile

contained in a thin-walled gall bladder, and under the influence of Myxosporidian infection the bile alters in colour and composition and the wall of the gall bladder becomes thickened.

TABLE 4.—*Trachinus trachinus*. (= ? *T. vipera*).

No. of fish	Degree of infection with Myxosporidia	Condition of bile	Condition of gall bladder	Other remarks
1	Uninfected	Very green, thin	Large, thin	Large mature ♀
2	"	"	"	" " "
3	"	Slight yellowish tint to the green	Small, thin	" " "
4	Early infection	Yellowish	Thick and very tough walled	Mature ♀, 26 cm. long
5	Uninfected	Bright green	Thin	Mature ♀, 28 cm. long

Thus, in 80 per cent. of the fishes examined, the bile was bright green and the walls of the gall bladder thin, and no *Myxosporidia* were present in the gall bladder or bile. Thickening of the wall and alteration in the colour of the bile coincided with the presence of parasites.

TABLE 5.—*Trachinus draco*.

No. of fish	Degree of infection with Myxosporidia	Condition of bile	Condition of gall bladder	Other remarks
1	Uninfected	Thin, green	Thin walled	
2	"	Yellowish green	"	Cestode larvae present in gall bladder

Uninfected bile appeared to be green. The presence of Cestode larvae in the gall bladder had caused a bile reaction somewhat resembling that produced in other fishes by the presence of *Myxosporidia*.

TABLE 6.—*Lepadogaster bimaculatus*.

No. of fish	Degree of infection with <i>Myxosporidia</i>	Condition of bile	Condition of gall bladder	Other remarks
1	Moderate	Yellow, slightly turbid	Thin	Very few of these fish were available for examination.
2	Slight	Yellow, thin	"	
3	Early infection, slight	Yellow, slightly turbid	"	
4	Uninfected	Green	Very thin	

While the gall bladders of the *Lepadogaster* were thin when infected, they were thicker and less transparent than those of uninfected fish. The bile also was yellow and thicker than that of the normal fish.

TABLE 7.—*Blennius ocellaris*

One specimen of this fish only was examined. It contained a *Myxidium* in its gall bladder, which was thin and had greenish yellow bile. The degree of infection was slight.

The tribe *Jugulares* of the *Acanthopterygii* as judged by *Trachinus trachinus*, *Trachinus draco*, *Lepadogaster bimaculatus*, and *Blennius ocellaris*, apparently undergo digestive derangements as a result of the presence of *Myxosporidia* in the gall bladder, the parasites producing alterations in the contents of the gall bladder.

TABLE 8.—*Cottus bubalis*.

No. of fish	Degree of infection with <i>Myxosporidia</i>	Condition of bile	Condition of gall bladder	Other remarks
1	Uninfected	Thin, green	Thin	Few trypanosomes in blood.
2	"	Vivid green	"	
3	Moderate infection	Faint greenish yellow	Thicker walled	

Cottus bubalis, family *Cottidae*, of the tribe *Scleroparei*, was the only member of the tribe available for examination. The presence of *Myxosporidia* in the gall bladder was shown by a change of colour of the green bile to yellow, and an increase in thickness in the wall of the gall bladder.

TABLE 9.—*Callionymus lyra*.

No. of fish	Degree of infection with Myxosporidia	Condition of bile	Condition of gall bladder	Other remarks
1	Very slight infection	Pale, yellowish green	Thin	Fish 13 cm. long
2	Heavy	Thick, orange	Very thick, opaque	16 cm. long
3	Uninfected	Yellowish green	Thin	12.8 " "
4	"	"	"	22.5 " "
5	Fairly heavy	Viscid, orange	Thick walled	12 " "
6	"	"	"	14.5 " "
7	"	"	"	13.5 " "
8	Uninfected	Thin, orange bile, but apparent fatty degeneration of liver	Thin	13.5 " "
9	Heavy	Blue-black	Thick	13.5 " "

Two-thirds of the *Callionymus* examined were parasitised and underwent profound changes in bile and gall bladder as a result. The *Gobiidae*, a family of the *Gobiiformes*, when parasitised thus, behave much as do other families among the *Acanthopterygii*.

TABLE 10.—*Solea vulgaris*.

No. of fish	Degree of infection with Myxosporidia	Condition of bile	Condition of gall bladder	Other remarks
1	Uninfected	Very green, thin	Thin walled	Very few trypanosomes in blood.
2	"	Green	"	
3	"	"	"	

The sole, as a representative of the *Pleuronectidae*, family of the tribe *Zeorhombi*, when normal showed green bile contained in a thin-walled gall bladder. The condition if *Myxosporidia* were present could not be determined, as material was lacking. Some normal plaice resembled the normal soles in the condition of their bile and gall bladder.

TABLE 11.—*Carassius auratus*.

No. of fish	Degree of infection with Myxosporidia	Condition of bile	Condition of gall bladder	Other remarks
1	Uninfected	Bright green	Thin walled	All these fish were 9.5 cm. long and came from the same hatch. They were examined for purposes of comparison and experiment.
2	"	"	"	
3	"	Green	"	
4	"	Green, very fluid	"	
5	"	Green	"	
6	"	"	"	
7	"	"	"	
8	"	"	"	

The *Ostariophysi* as represented by the goldfish (*Carassius auratus*) agree with most other Teleosts in possessing green bile.

It may be mentioned that goldfish, from the same stock,

artificially infected with *Myxosporidia*, had yellowish bile and thickened gall bladders.

TABLE 12.—*Scomber scomber*. (= *S. scombrus*.)

No. of fish	Degree of infection with <i>Myxosporidia</i>	Condition of bile	Condition of gall bladder	Other remarks
1	Uninfected	Yellowish red, thin	Thin walled	Fish 20 cm. long
2	"	"	"	24 cm. long
3	"	"	"	24 " "
4	"	"	"	15 " "
5	"	"	"	20 " "
6	Infected	Red to blood-red	Slightly thicker walled	24 " "
7	Uninfected	Blood colour, thin	Thin	30 " "
8	"	"	"	24 " "
9	"	"	"	24 " "
10	"	"	"	22 " "
11	"	"	"	25 " "
12	"	"	"	27 " "
13	"	"	"	29 " "
14	"	"	"	25 " "
15	Slight infection	Blood colour, thin, small quantity	Slightly thicker walls	28 " "
16	Uninfected	Thicker, yellowish red	Thin	28 " "
17	Infection moderate	Abundant bile, fairly fluid	"	30 " "
18	"	Small quantity, red, fluid	"	27.5 " "
19	Uninfected	Blood colour, thin	"	29.5 " "
20	"	"	"	24 " "
21	"	"	"	24 " "
22	"	"	"	24 " "
23	"	"	"	24 " "
24	"	"	"	24 " "
25	"	"	"	24 " "

Scomber scomber—the mackerel—the representative of the *Scombriformes* examined, differs from the other families so far recorded, in that its bile coloration showed little or no difference

when *Myxosporidia* were present. Its colour is, then, not markedly associated with Myxosporidian infection, and neither the colour nor the extremely slight thickening of the walls of the bladder were of use as rough criteria of the infected or normal conditions.

Among the *Apoda*, both *Anguilla* and *Conger* were examined. The bile was green in all the cases, and no *Myxosporidia* were found in the clear, transparent gall bladders.

Ammodytes tobianus afforded an example of the *Percesoces*, but proved uninfected, though a fair number were examined.

(B) ELASMOBRANCHII

Among the *Selachii*, members of the sub-orders *Squali* and *Raji* were examined, to see whether the same conditions obtained as in the *Teleostei*.

TABLE 13.—*Selachii*.

Name of fish	No. of fish	Degree of infection with <i>Myxosporidia</i>	Condition of bile	Condition of gall bladder
<i>Scyllium catulus</i>	1	Uninfected	Greenish	Thin
<i>Galeorhinus galeus</i> (= <i>Galeus canis</i>)	1	Slight infection	Greenish, limpid	Slightly thickened
”	2	Heavy infection	Orange, much mucus—like table-jelly	”
”	3	Uninfected	Green	Thin
<i>Acanthias vulgaris</i>	1	Moderate infection	Yellowish	Thick
<i>Raja batis</i>	1	Uninfected	Thin, green	Thin
”	2	”	”	”
”	3	”	”	”
”	4	”	”	”
<i>Raja maculata</i>	1	Moderate infection	Pale yellow	Slightly thickened
”	2	Uninfected	Greenish	Thin
”	3	”	”	”
”	4	”	”	”

As with the *Teleostei*, so with the *Selachii*, it seems, so far as our experience goes at present, that the presence of *Myxosporidia* in the gall bladder produces some thickening and also changes in the colour and composition of the bile.

(C) PATHOLOGICAL EFFECTS

As most previous workers on the *Myxosporidia* occurring in piscine gall bladders have considered that the free floating parasites usually have little or no direct effect upon the host, we wish to record very briefly the results of our preliminary examination of preparations of the *Myxidium*-infected gall bladders of *Gadus pollachius*.

(a) There is a leucocytic infiltration around the small blood vessels in the fibro-muscular coat of the infected gall bladder.

(b) The leucocytic infiltration is especially pronounced in some places in the sub-serous layer, and in the immediately adjacent portions of a peculiar tissue which covers part of the outside of the gall bladder. This tissue has, according to Dr. Seidelin, a glandular structure and represents, in his opinion, pancreas.

(c) The leucocytic infiltration is also considerably increased at the bases of some of the papillae of the mucosa. In connection with this leucocytic infiltration there appears to be a considerable number of young fibroblasts, but it is difficult to state whether the presence of such is a normal occurrence or not.

These leucocytic infiltrations indicate a condition of inflammation of the gall bladder.

(d) Desquamation of the epithelium of the mucosa occurs in some places, and degenerating epithelium is found in the bile.

(e) Numerous mucous cells are found at various points in the epithelium. The distribution of such areas is irregular.

(f) There is no doubt that the *Myxosporidian* parasites are adherent to the mucosa. In some cases, processes from *Myxosporidian* trophozoites can be seen clearly penetrating the epithelium of the gall bladder. These parasitic intrusions have been seen by other observers to whom we have shown our sections.

(g) Regarding the possibility of small intra-epithelial stages of the parasites, we have seen certain structures—and so have some of

our colleagues—which could be interpreted as such. These intra-epithelial bodies seem identical with those figured by Auerbach (1910)* in the case of *Myxidium bergense*, parasitic in *Gadus virens*. We prefer, however, to defer a definite pronouncement on this difficult matter.

(*h*) In fine, it would appear that the presence of Myxosporidia is apt to irritate the gall bladder, leading to a catarrhal secretion of mucus and desquamation of epithelium, and to inflammatory conditions throughout the wall. The increase of the mucus accounts for the viscosity of the contents of many infected gall bladders. To some extent the discoloration of the bile is probably due to the presence of parasites.

The existence of a catarrhal cholecystitis seems to explain satisfactorily the described alterations in consistence and colour of the contents of infected gall bladders.

We have much pleasure in thanking Dr. Seidelin and Dr. G. C. Simpson for examining our sections and for suggestions used in writing this sub-section.

It should be mentioned that the results previously tabulated, both gross and pathological, appeared to be in no wise different whether the fish in question had fed recently or was fasting. In every case there was sufficient bile present for determination of its colour and consistency, even though the wall of the gall bladder were thickened. Empty gall bladders were not found in any of the fish examined. It may be of interest to add that most of our results were obtained in the month of August, 1912.

Some effects of Myxosporidia on other organs of fish with biliary infection are briefly recorded in Section V, page 481.

Parenthetically, we may mention that, although anxious to give as correctly as possible the names of the piscine hosts for purposes of reference, we have had some difficulty in certain cases. English and French authorities differ on the classification and nomenclature of fishes. Hence the use of synonymous names in various parts of this paper.

* 'Die Cnidosporidien,' p. 105. Leipzig: Werner Kleinhardt.

IV. TABULAR SUMMARY

To complete the survey of the distribution of the parasites in the gall bladders of various fishes, together with the effects observed, we may tabulate the results obtained thus:—

TABLE 14.—*Teleostei*.*

Sub-order	Tribe	Family	Genus	General result	
Apodes		Anguillidae	Anguilla	Examination negative. Effect of Myxosporidia undetermined. Normal bile green.	
			Conger		
Percesoces		Ammodytidae	Ammodytes	Fish uninfected, bile green.	
Anacanthini		Gadidae	Gadus (3 spp.)	Normal bile green, gall bladder thin; infected bile yellow, gall bladder thick.	
Acanthopterygii	Scombriformes	Scombridae	Scomber	Normal and infected bile much alike, gall bladder slightly thicker if infected.	
	Zeorhombi	Pleuronectidae	Solea	Normal bile green.	
	Gobiiformes	Gobiidae	Callionymus	Normal bile green, and gall bladder thin. Infected bile thick, orange to blue-black, gall bladder thickened.	
	Scleroparei	Cottidae	Cottus	Normal bile green, gall bladder thin. Infected bile yellowish, gall bladder thicker.	
	Jugulares		Trachinidae	Trachinus (2 spp.)	Normal bile green, thin; gall bladder thin. Infected bile yellowish, gall bladder thicker.
			Gobiesocidae	Lepadogaster	Normal bile green, infected yellow. Normal gall bladder thin, infected thicker.
			Blenniidae	Blennius	Normal bile green, thin; infected bile yellowish, thicker; gall bladder thicker when infected.
Ostariophysi		Cyprinidae	Carassius	Normal bile green, thin; infected bile yellowish.	

* Classification based on that given in Sedgwick's 'Zoology,' Vol. II (1905).

TABLE 15.—*Elasmobranchii*.

Order	Sub-order	Family	Genus	General result
Selachii	Squali	Scylliidae	Scyllium	Normal bile greenish.
		Spinacidae	Acanthias	Infected bile yellow, gall bladder thick.
		Carchariidae	Galeus	Normal bile green, thin; infected bile very thick, gall bladder thickened if infected.
	Raji	Rajidae	Raja (2 spp.)	Normal bile green, thin; infected bile thicker, yellow; gall bladder slightly thickened if infection present

From the 'distribution' summary it is seen that in a fairly wide range of fishes the presence of *Myxosporidia* in the gall bladder is associated with thickening of the organ and with changes in the contents.

It may be of interest to note that green bile removed from a freshly-killed fish remained green for as long as a couple of days, while the discoloured bile in an infected fish was obviously discoloured at the moment of the death of the fish. Decapitated fish, whose muscles still executed vigorous movements, if infected, had discoloured bile. In other words, the discoloration of the bile was certainly not a post-mortem effect.

Direct experiments showed that the change in colour was induced by the Myxosporidian parasite. *Gadus luscus* was never found naturally infected with *Myxosporidia*. But two *Gadus luscus* artificially infected by way of food showed yellowish colour of the bile when killed a few days later, and in the bile there were found young stages of the parasite administered to them. The control fish had clear, green, parasite-free bile.

V. OTHER EFFECTS

Other effects of Myxosporidian infection also briefly noted were as follows:—

Infected *Gadus pollachius* had larger livers and gall bladders than normal *G. pollachius* of the same length. Those containing *Myxosporidia* were thinner as a rule than uninfected specimens of the same length. An infected male was thinner than a normal male, and the same was true for the females.

In the case of *Scomber scomber*, the bile, when normal, contained a fair amount of fat. Infected bile showed much less fat.

Acute inflammation of the intestine was sometimes noticed in infected fish.

In some cases the condition of the bile may have hindered pancreatic digestion also, for in a few fish the bile gave an acid reaction instead of the usual alkaline one. The interference with pancreatic digestion would probably, in part, explain the thinner musculature of the body of an infected fish.

VI. CONCLUDING REMARKS

It is obvious, then, that biliary Myxosporidiasis has an economic as well as a biological interest, and hence may be added to the catalogue of little-known diseases well worthy of more detailed investigation. Fish which appeared healthy externally often showed, on closer examination, slight emaciation when Myxosporidia were present. We hope to deal with some of the Myxosporidian parasites in greater detail shortly, and, if possible, to discuss more fully the various other changes occurring in infected fish.